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PRINCIPAL INVESTIGATOR: Nadine Hempel, PhD

CONTRACTING ORGANIZATION: The Pennsylvania State University
Hershey, PA 17033

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6. AUTHOR(S)
Nadine Hempel, PhD; Dong Hui Shin, PhD

E-Mail: nhempel@psu.edu

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The Pennsylvania State University
500 University Drive, P.O.
Box 850, MC H138
Hershey, PA 17033-0850

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14. ABSTRACT

Our work addresses the hypothesis that mitochondrial dysfunction plays a role in the etiology and chemoresistance of epithelial ovarian cancers. We are focusing on the role of the fission protein Drp1 in this context. Specifically we discovered that expression of a low molecular weight Drp1 variant is associated with mitochondrial fission/fusion defects. Mass spec and RNA sequencing analysis has revealed that the low molecular weight (LMW) isoform of Drp1 does not arise as a consequence of alternate transcriptional promoter use, but may be dependent on an alternate variable domain and C-terminal truncation. We are interrogating the role of short Drp1 as a dominant negative fission protein and are investigating its binding affinity to mitochondria and interaction with fission accessory proteins. Investigations on the function of this protein in mediating mitochondrial dysfunction and chemoresistance are ongoing. We have identified that expression of LMW Drp1 is detected in the majority of high grade serous ovarian cancer cells isolated from patient ascites, and that this is associated with hyperfused mitochondria, indicating that this is a clinically relevant observation that could affect a majority of ovarian cancer cases.

15. SUBJECT TERMS
Drp-1, DNM1L, mitochondria, mitochondrial fission, ovarian cancer

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1. INTRODUCTION:

Epithelial Ovarian Cancer (EOC) remains the most deadly gynecological malignancy, characterized by high rates of relapse and chemoresistance. Metabolic screening of EOC cell lines and patient ascites-derived tumor cells revealed that a distinct subgroup of EOC demonstrate severe mitochondrial dysfunction, which is accompanied by hyperfused mitochondria and expression of a low molecular weight variant of the mitochondrial fission protein Drp1. Our data suggest that compromised mitochondrial function and fission/fusion dynamics may be a hallmark of a previously unidentified subgroup of highly chemoresistant EOCs and that this is associated with aberrant expression of the fission protein Drp1. This work addresses the hypothesis that Drp1-dependent mitochondrial dysfunction plays a role in the etiology and chemoresistance of a distinct subgroup of epithelial ovarian cancers. Our major aims of this work are to 1. establish the clinical significance of mitochondrial fission dysfunction related to Drp1 expression in a cohort of ovarian cancer patients, 2. elucidate the mechanistic consequences of Drp1 splice variant expression on EOC mitochondrial function, metabolism and chemoresistance, and 3. investigate alternate therapeutic strategies for chemoresistant mitochondria-deficient EOCs. These studies have implications for the future development of Drp1 variants as biomarkers in the screening and identification of a subgroup of highly chemoresistant EOCs. The reliance of these cells on alternate metabolic pathways as a consequence of their mitochondrial deficiency makes this subgroup of EOCs a target for anti-metabolism based therapies.

2. KEYWORDS:

Drp-1, DNM1L, mitochondria, mitochondrial fission, ovarian cancer

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The major goals of the project are listed below in the format of the original SOW:

Specific Aim 1: To establish clinical significance of mitochondrial dysfunction in EOC

- **Major Task 1** Test if mitochondrial dysfunction is a common clinical phenotype of highly chemoresistant EOCs:
    - *Milestone #1: IRB and HRPO approval received – 100% completed.*
        - Subtask 1: Obtain IRB approval & submit necessary documents for HRPO review. IRB (STUDY4648; 03-29-2016) and HRPO (A-19540; 29-07-2016) approval received
    - *Milestone #2: Completion of statistical analysis of patient mitochondrial dysfunction and association with clinical parameters – 100% established techniques and methods, 40% completed analysis of patient EOCs, anticipated completion following recruitment of additional patients to the study.*
        - Subtask 2: Isolation and culturing of cells from patient ascites.
        - Subtask 3: Statistical Analysis patient data: - to be completed following collection of all patient samples.
- **Major Task 2** Determine the identity and frequency of expression of Drp1 splice variants, and examine their association with mitochondrial dysfunction and chemoresistance.
    - *Milestone #3: Major Drp1 splice variant in mitochondrial deficient EOCs identified – 100% completed.*
        - Subtask 1: Identified Drp1 splice variants by mass spec.

        - Subtask 2: Identified Drp1 splice variants by RNA Seq and 3'RACE.
        - Subtask 3: Design primers for identification of Drp1 splice variants in patient specimens.
    - *Milestone #4: Completion of statistical analysis of patient mitochondrial dysfunction and association with Drp1 expression – 100% established techniques and methods, 40% completed analysis of patient EOCs, anticipated completion following recruitment of additional patients to the study.*
        - Subtask 4: Assess Expression of Drp1 variants in patient derived EOC RNA samples.
        - Subtask 5: Assess mitochondrial morphology and chemoresitance of patient derived EOC RNA samples.
        - Subtask 6: Statistical Analysis of patient data, association of Drp1 expression with mitochondrial function, chemoresistance profiles and histological subtype .
- **Major Task 3** Test if DNM1L copy number alterations correlated with Drp1 expression
    - *Milestone #5: Completion of all data analysis of clinical specimen – 100% established techniques and methods, 40% completed analysis of patient EOCs, anticipated completion following recruitment of additional patients to the study.*
        - Subtask 1: Assess DNM1L copy number in patient derived EOC RNA samples.
    - *Milestone #6: Manuscript submission of data on mitochondrial function and & association with Drp1 expression & DNM1L copy number alterations – in preparation, to be completed after collection of additional patient samples*

Specific Aim 2: To elucidate the mechanistic consequences of Drp1 splice variant expression on EOC mitochondrial function, metabolism and chemoresistance.

- **Major Task 4** Test if identified Drp1 transcript variants act as dominant negative fission proteins.
    - *Milestone #7: identification of Drp1 variant function on mitochondrial fission and function – 90% completed.*
        - Subtask 1: Cloning of Splice variants.
        - Subtask 2: Experiments of recombinant Drp1 constructs on mitochondrial fission using confocal microscopy & TEM & Seahorse extracellular flux analysis.
    - *Milestone #8: identification of Drp1 splice variant protein-protein interaction - 90% completed.*
        - Subtask 3: protein-protein interaction studies.
- **Major Task 5** Determine if Drp1-mediated mitochondrial dysfunction aids in chemoresistance by disrupting programmed cell death.
    - *Milestone #9: Identification of Drp1 variant expression on programmed cell death and chemoresistance and finalization of data analysis – 80% achieved*
        - Subtask 1: Effects of recombinant Drp-1 constructs on apoptosis & autophagy markers in response to chemotherapeutics.
        - Subtask 2: Effects of recombinant Drp-1 constructs on Bax interaction.

- *Milestone #10: Manuscript submission of data Drp1 variants as potential dominant & negatives in the regulation of mitochondrial fission and programmed cell death – Manuscript in preparation.*

- **Major Task 6** Assess if Drp1-mediated mitochondrial dysfunction contributes to chemoresistance by eliciting cellular DNA damage response.
  - *Milestone #11: Identification of Drp1 variant expression on DNA damage response and chemoresistance & finalization of data analysis – 50% completed*
    - Subtask 1: Effects on ECCR1 protein stability in response to recombinant Drp1 variant expression & assessment of Drp1 expression and other DDR protein levels.
    - Subtask 2: effects of recombinant Drp1 splice variants on DDR signaling.
    - Subtask 3: effects of ATM/ATR inhibitors on Drp1 variant expressing cell lines and EOC specimen.
  - *Milestone #12: Manuscript submission of data Drp1 variants as potential dominant negatives in the regulation of mitochondrial fission and programmed cell death – in preparation*

Specific Aim 3: To investigate alternate therapeutic strategies for mitochondria-deficient EOCS

- **Major Task 7** Determine the alternate metabolic pathways used by mitochondria defective cells.
  - *Milestone #13: correlation of metabolic inhibitor response with EOC metabolic profiles and mitochondrial dysfunction - 70% completed*
    - Subtask 1: Growth studies of EOCs under nutrient limitations.
    - Subtask 2: Glycolytic flux/Bioenergetics measurements of EOC specimens.
- **Major Task 8** Assess if disruption of alternate metabolic pathways by metabolism-based inhibitors represents a novel therapeutic strategy to target mitochondria-defective cells.
    - Subtask 1: Dose response curves to metabolic inhibitors Ovca cell lines
    - Subtask 2: Metabolic inhibitor studies on cell viability of EOC specimens
  - *Milestone #12: Manuscript submission describing the relevance of metabolic targeting of mitochondria defective EOCs – in preparation.*
  - *Milestone #13: Submit grant application to NCI R01 for follow up work – 100% achieved, R01 submitted Jun 2017; resubmission: October 2018.*

What was accomplished under these goals?

Activities & Outcomes:

- We continue to collect patient ascites samples and have identified that expression of LMW Drp1 is primarily expressed in cells of high grade serous adenocarcinoma origin (HGSA, Figures 1A & B), with 50% of HGSA samples displaying expression of LMW-Drp1 that exceeds expression of full length Drp1 (Figure 1B). Strong expression of LMW-Drp1 correlates with hyperfusion and aggregation of mitochondria (Figure 1C).
- Ues of two different antibodies allows detection of Fl and LMWDrp1, due to their epitope localization in the Cterminal truncation site (Figure 1D)
- Using Mass spec analysis we confirmed that the low molecular weight (LMW) 60kDa variant is Drp1 and has a truncation in the C-terminal domain (Figure 1E & F).

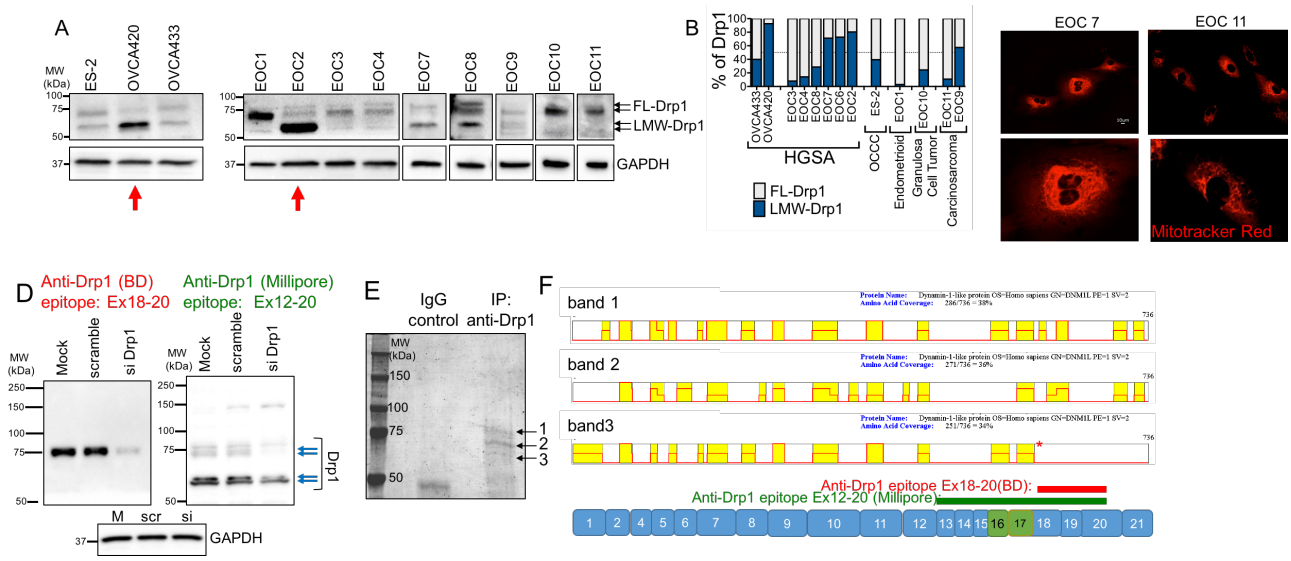

Figure 1: **A.** LMW Drp1 protein expression in cell lines (OVCA420, OVCA433 & ES-2) and patient ascites derived epithelial ovarian cancer cells (EOC, western blotting). **B.** Ratio of full length (FL) to Low Molecular Weight (LMW)-Drp1 expression in cell lines and EOC samples, grouped by histological classification. **C.** EOC7 cells expressing LMW-Drp1 demonstrate hyperfused mitochondria compared to EOC11 cells, which predominantly express FL-Drp1 (Mitotracker CMX-ROS staining). **D.** FL-Drp1 but not LMW-Drp1 is detected by an antibody targeting the C-terminus (BD-epitope exon 18-20) **E&F.** Mass Spec analysis of Drp1 variants was carried out following IP with Drp-1 polyclonal antibodies (Wistar Mas Spec core) and confirmed the presence of Drp1 peptide sequences in all three bands. Band 3 is LMW Drp1. A lack of coverage in the C-terminal domain suggests C-terminal truncation in the LMW variant. Alternately spliced Exons 16 and 17 are located in the variable domain responsible for Drp1 intermolecular interaction.

- RNA seq, RT-PCR and 5'RACE analysis was performed to determine if this variant arises as a consequence of alternate 5'promoter use or alternate splicing. Using 5'RACE we determined that alternate promoter use likely does not contribute to the generation of a shorter transcript, which was confirmed by RNA seq data. Lack of alternate start sites were further confirmed by Mass Spec analysis.
- 3'RACE was carried out to determine the exact sequence identity of the Drp1 C-terminal truncation in OVCA420 and OVCA433 cells (Figure 2A). Following 3'RACE, PCR products were cloned and sequenced. In addition to full length Drp1 transcript, 3'RACE PCR products contained three different C-terminal truncation transcript variants: ending in either exon 14 (ΔC-Ex14), exon 15 (ΔC-Ex15), or in exon 17, with an additional 16 amino acids, novel STOP codon and termination signals (ATTAAA; ΔC-Ex17). ΔC-Ex14 and ΔC-Ex17 were exclusively isolated from RNA of OVCA420 cells, while ΔC-Ex15 was found in both cell lines. In addition, we identified that ΔC-Ex17 predominatly includes both Exon 16 &17 (Figure 2B).

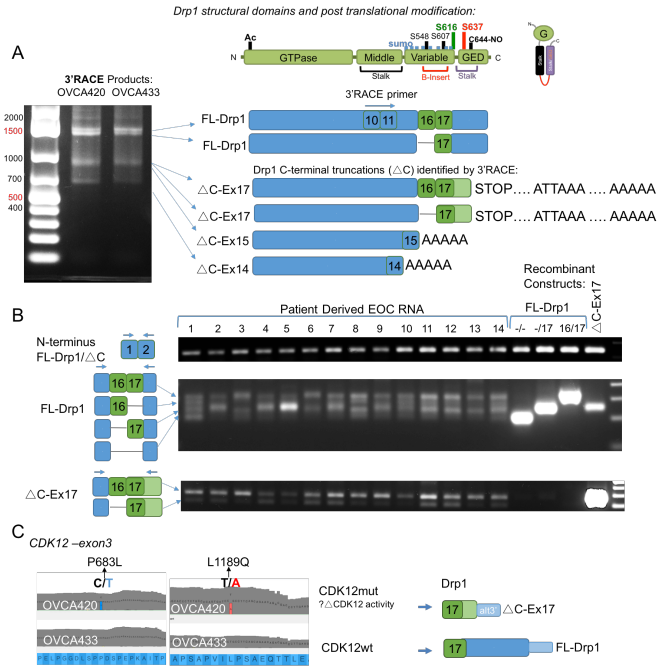

Figure 2: **A.** 3'RACE identified several C-terminal truncation transcripts in OVCA420 and OVCA433 cDNA including full length transcripts with variable exon 16 and 17 splicing. **B.** RT-PCR specific to the variable region of Drp1 identifies alternate splicing of Exon 16 and 17 and expression of ΔC-Ex17 in patient rderived specimens (EOC) derived from ascites and cultured in vitro prior to RNA isolation. Ratio of full length (FL) to Low Molecular Weight (LMW)-Drp1 expression in cell lines and EOC samples, grouped by histological classification. **C.** OVCA420 cells have Exon 3 and 13 mutations in one allele of *CDK12*, resulting in AA substitutions near the kinase domain. This kinase could be responsible for Drp1 Alternate last exon splicing. CDK12 mutations were identified by RNAseq.

- We also discovered that differential splicing of exon 16 and 17 of the "variable" region of Drp1 is a common phenotype of ovarian cancer cells. This region is of interest has it has been implicated with intermolecular Drp1 binding and binding of Drp1 to mitochondria via mitochondrial receptors such as Drp1 (Figure 2B). Using RNAseq, and a series of variant specific RT-PCR conditions we further identified the pre-dominant Drp1 transcript variants in 15 patient specimens. From this we could conclude that there is heterogeneity in Drp1 variant expression: The major forms of full length Drp1 include either both exon 16 and 17 or exon 17 only. This has consequences for Drp1 localization in ovarian cancer cells as missing exon 16 has been associated with enhanced microtubule binding during mitosis.
- The existence of a C-terminal Drp1 truncation (ΔC-Drp1) and alternate splicing patterns of Exons 16 and 17, suggests that alternate last exon splicing is specifically involved in generating different 3' truncated Drp1 transcripts. In an effort to assess if there may be inherent differences in splicing factors in our cell lines, we looked to CDK12, which is mutated in some HGSAs (TCGA). CDK12 mutations result in disruption of its kinase activity and this dysregulates DNA repair protein expression in ovarian cancer. Besides it's role in phosphorylating the C-terminal repeat domain of the RNA Pol II complex (Bartkowiak et al 2010), CDK12 was recently shown to have novel functions as a component of spliceosomes (Ekumi 2015; Liang et al, 2015). Importantly, CDK12 has a specific role in alternate last exon splicing(Tien et al, 2017). Intriguingly, OVCA420 cells display CDK12 mutations that lead to amino acid substitutions flanking the kinase domain

(Figure. 2C), and we confirmed that EOC samples from patient ascitites have various CDK12 mutations, as determined by RNA seq. Interestingly the frequency of observed mutations was higher than reported by TCGA, with 70% of samples displaying mutations in CDK12. We hypothesize that a loss/decrease in CDK12 activity due to some of these mutations may result in preference for alternate splicing of shorter *DNM1L* transcripts. This is in line with the report that knock-out of CDK12 results in the preference of the shorter 3' transcripts of genes that display alternate last exon splicing, with a ATM transcript variant expression being a recently described example (Tien et al, 2017).

- Moreover, we discovered that LMW-Drp1 was primarily expressed in cells grown under attached proliferating conditions, and that full length Drp1 expression was restored when cells were grown in anchorage independent conditions (Figure 3A). This was accompanied by increased fission in anchorage independent spheroid conditions, as demonstrated by shorter mitochondrial fragments in TEM and with increased mito/autophagy, as demonstrated by LC3B western and autophagosomes visible by TEM (Figure 3B&C). We are currently investigating if autophagy leads to increased expression of FL-Drp1 and suppression of the short Drp1 variants.

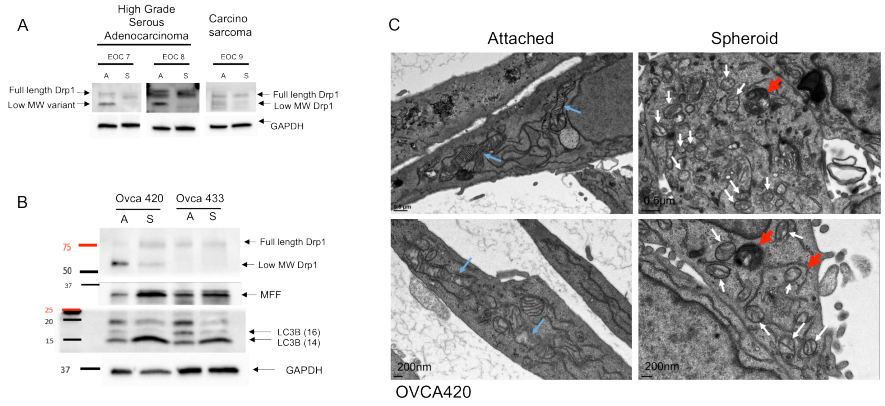

Figure 3: Strong expression of LMW Drp1 is observed in patient ascites derived tumor cells from HGSA histological subtypes (**A**). LMW Drp1 expression is decreased and FL Drp1 expression increased when cells are grown in anchorage independent spheroids (S) in Ultra low attachment plates for 3 days, compared to attached conditions (A). Increased FL Drp1 expression is associated with autophagy markers LC3B (**B**) and shorter mitochondria as visualized by TEM (**C**). Blue arrows indicate elongated and hyperfused mitochondria in OVCA420 cells in attached conditions, while arrows indicate smaller mitochondria in spheroid conditions. Red arrows indicate auto phagosomes.

- The above results have provided us with novel avenues to pursue the regulation of Drp1 variant expression in ovarian cancer that will be addressed in our R01 application (see Other goals achieved).
- Drp-1 CRISPR/Cas9 OVCA433 knockdown cell lines were generated (Figure 4) and verified by sequencing, which revealed that 2/3 copies of *DNM1L* gene were knocked out.

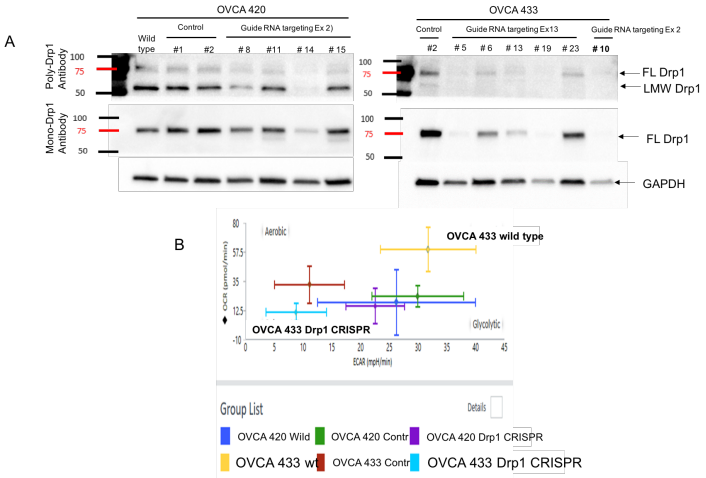

Figure 4: A Drp1 knock-out cells were generated using the CRISPR/Cas 9 system and the effects of Drp1 knock-down assessed by Seahorse extracellular flux analysis (**B**)

- Following identification of low molecular weight Drp1 variants (Aim 1) using 3'RACE, we set out to clone the identified Drp1 constructs into mCherry expression vectors. Drp1 CRISPR cell lines were used for re-expression of full length and Low molecular weight variants. Cell lines stably expressing various Full length Drp1 constructs (different combinations of alternative spliced exon 16 & 17) and newly generated Drp1 truncation variants (ΔC) ending in Ex 14, Ex15 and Ex17 were generated in these CRISPR knock-down cell lines.
- First we investigated if these have consequences on ovarian cancer cell line mitochondrial fission. Re-expression of full length Drp1 in OVCA420 rescued their mitochondrial aggregation phenotype, unlike a dominant negative Drp1 construct (Figure 5A).
- Using mitotracker and immunofluorescence imaging we have shown that Drp1 mislocalizes to the cytoplasm in OVCA420 cells, suggesting that LMW Drp1 influences Drp1 binding to mitochondria (Figure 5B&C).
- co-IP studies to determine interaction of LMW-Drp1 with the mitochondrial receptor, Mff, suggest that LMW-Drp1 does not bind Mff (Figure 5D).
- Effects of Drp-1 C-terminal variant expression on mitochondrial morphology was assessed by mitotracker co-staining, revealing that ΔC-Ex14 & ΔC-Ex15 Drp1 truncations did not localize to mitochondria, while ΔC-Ex17 had lower mitochondrial association than full length Drp1 (Figure 5E). As expected, FL-Drp1 (x/x) and (16/17) associated in small puncta at the ends of mitochondrial fission points. Moreover, some aggregation of FL-Drp1 (16/17) was also observed in the perinuclear space that did not co-localize with mitochondria. Surprisingly, we found little mitochondrial localization of the full length construct lacking exon 16 (Fl-Drp1(x/17)). Intriguingly overexpression of this construct also decreased the colongenic potential of OVCA433 cells (Figure 6A).

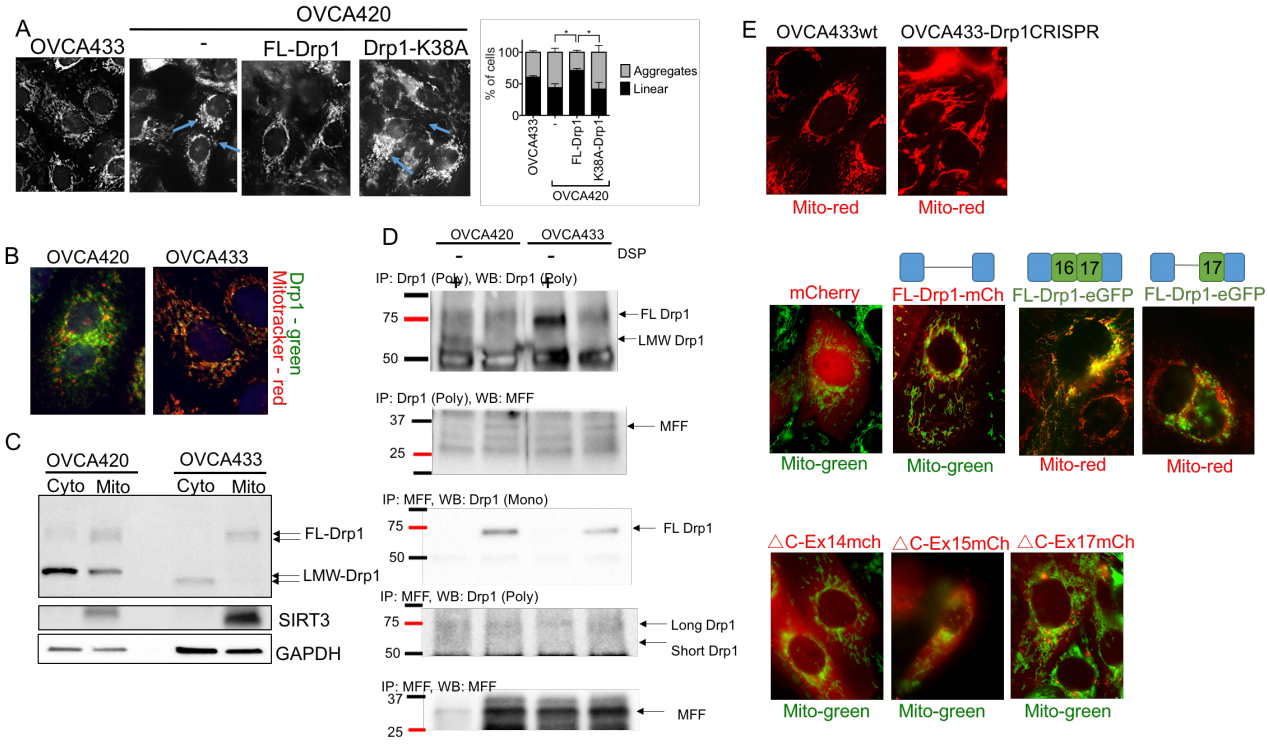

Figure 5: A. Drp1 expression restores fusion in OVCA20 cells & decreases the number of cells with mitochondrial aggregates. Cells were transfected with FL-Drp1 or dominant negative Drp1-K38A. Mitochondria were stained with Mitotracker & cells with aggregates counted (*p<0.05, ANOVA). **B.** Drp1 mis-localizes to the cytoplasm in OVCA420 cells. Immunofluorescence staining reveals punctate staining of Drp1 (C-termi. Specific antibody) at sites of mitochondrial fission in OVCA433 cells and localization to cytoplasm in OVCA420 cells. **C.** Localization of Drp1 variants is visualized by westerns following fractionation of lysates into cytosolic and mitochondrial fractions. LMW Drp1 is found in the cytoplasm and fails to associate with mitochondria at distinct fission puncta in OVCA420 cells when compared to OVCA433 cells which express predominantly FL Drp1. **D.** Mff interacts with FL Drp1, but not with LMW-Drp1, as assessed by co-IP. E. Localization of cloned Drp1 expression constructs and C terminal truncation variants. Constructs are either tagged with mCherry or eGFP and mitochondria visualized by either Mitotracker Green or Red reagent, respectively.

- Assessment of clonogenic potential revealed that expression of exon 16 in FL –Drp1 is important in sustaining clonogenic survival, and that expression of truncation mutants could rescue colony formation similar to FL-Drp1 (Figure 6A). These data demonstrate that expression of Drp1 C-terminal trunctation mutants is not detrimental to cellular viability.
- Cell viability assays in response to drug treatments revealed that expression of different Drp1 variants had no effect on response to cisplatin. However, compared to all other variants and wild type Drp1 constructs, ΔC-Ex17 and FL-Drp1 containing both Ex16 and 17 were able to increase the IC50 dose, suggesting that ΔC-Ex17 and FL-Drp1(16/17) might confer partial resistance to this microtubule targeting agent (Figure 6B). Interestingly, their mitochondrial localization was similar, both accumulating in perinuclear aggregates, and ΔC-Ex17 and FL-Drp1(16/17) represent the most highly expressed variants in our EOC cohort (Figure 2B).
- As predicted C-terminal truncation mutants were more sensitive to glucose deprivation than cell expressing FL-Drp1, suggesting that high expression of Drp1 truncation mutants leads to lack of mitochondrial fission and a switch to glycolysis (Figure 6C).

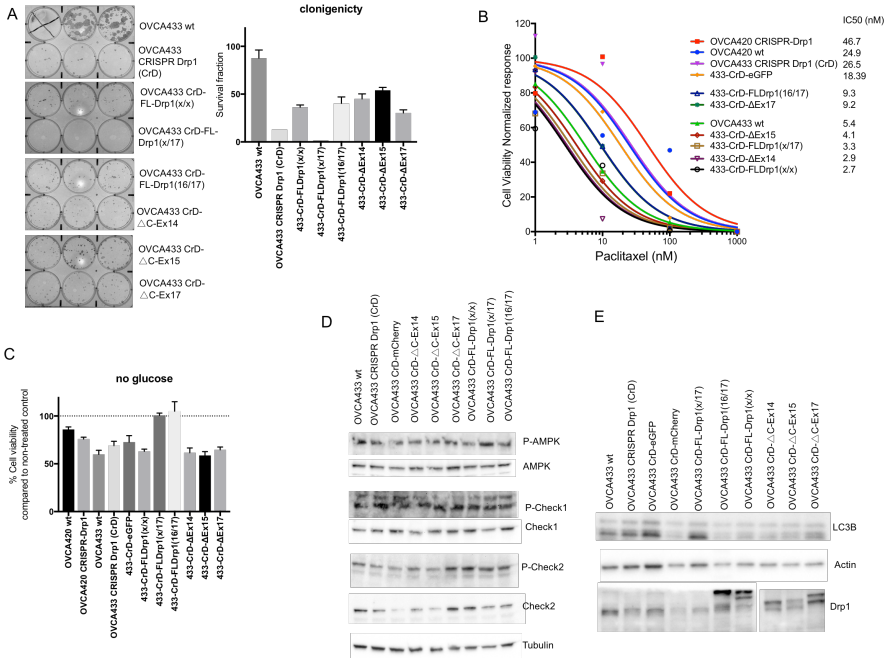

Figure 6: **A**. Clonogenic survival of OVCA433 following Drp1 CRISPR/Cas9 knock-down and reexpression of Drp1 transcript variants. **B**. Dose response curve of Paclitaxel treatment on OVCA433 cell viability. Cells were treated for 48hr in serum free RPMI. **C.** Cell viability following culturing of cells in glucose free media for 72hrs. **D**. Protein expression of DNA damage response proteins and AMPK in response to Drp1 variant expression, as assessed by western blotting. **E.** Protein expression of LC3B autophagy marker and recombinant Drp1 constructs.

- Interrogating the role of Drp1 on DNA damage response, we observed that knock down of Drp1 resulted in decreased Phospho and total Chek2 levels, and that re expression of all FL-Drp1 and ΔC-Ex17 were able to rescue this while ΔC-Ex14 & ΔC-Ex15 did not.
- We observed less autophagic LC3B signals in cells expressing the C-terminal truncation variant and FL-Drp1 constructs compared to expression of FL-Drp1(x/17) (Figure 6D). Since this FL Drp1 construct is expressed heterogeneously in our cohort of Ovarian cancer patients, we will further explore the role of this variant in Ovarian cancer biology in our future studies (proposed in R01 application, see below).

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Other goals achieved:

- A R01 grant entitled "Mitochondrial heterogeneity in metastatic ovarian cancer" in response to the NCI RFA provocative question PQ5: *How does mitochondrial heterogeneity influence tumorigenesis or progression?, was submitted in June 2017. It received a priority score of 48.*
- Data gathered by funding under the current award in Year 2, has aided in our preparation of the A1 revision of the above R01 application, to be submitted October 29, 2018 in response to the same RFA.
- A new area of investigation developed as a consequence of our access to patient derived EOCs and ascites fluid. Using ascites fluid as conditioned media we discovered that this fluid provides a reducing environment for cancer cells to aid their survival in response to redox stress. We are now investigating the role of the antioxidant enzyme GPX3, in manipulating the extracellular redox environment and ascites in ovarian cancer. We are in final stages of preparing a manuscript of these findings.
- A new area of investigation emanating from this work is our discovery that Drp1 transcript variants are dynamically regulated during different stages of metastasis. This has prompted us to look further at the regulation of the Drp1 transcripts in relation to their regulation by miRNAs and alternate splicing. This is included in our R01 revision application to the NIH.

Stated goals not met: We have mostly kept on track with experiments based on our Milestones of the SOW. Milestones that are 80-90% completed primarily require extra replicate experiments for the finalization of the manuscripts. Some delays were experienced in getting RNASeq and Mass spec data, due in part to trouble shooting of purification and preparation of proteins for mas spec analysis and turn-around time in obtaining data for RNA seq and mass spec from core facilities. Delays were also experienced in generating Drp1 CRISPR/Cas9 cells and with troubleshooting the cloning procedure for the ΔC constructs. The latter being due to our unexpected discovery that EOC specimens contain a variety of FL and ΔC transcripts, some of which required complex cloning strategies (e.g. ΔC-Ex17). In addition to a 4 month delay in getting IRB and HRPO approval, we experienced a lower recruitment of patients to the study than anticipated, and have thus far collected 12 ascites specimens during the 2 year period. This has delayed reaching milestones of Aim 1, as we are continuing to collate data on additional patient samples expected in the coming year. We will use the to detect Drp1 variants in these specimens. However, in Year 2 we have established all methods developed above to enable us to quickly analyze our data once our patient quota has been reached.

What opportunities for training and professional development has the project provided?

- The PI, Dr. Nadine Hempel, and postdoc, Dr. Dong Hui Shin, attended the *NIH special conference on mitochondria in May 2016* in Bethesda MD. At this meeting both were exposed to the most recent developments and cutting edge research techniques used in mitochondrial research.
- Dr. Hempel attended the *Marsha Rivkin symposium on Ovarian cancer* in September 2016, Seattle WA to gain exposure to the latest developments in ovarian cancer research.
- The PI, Dr. Nadine Hempel, and postdoc, Dr. Dong Hui Shin, attended the *Society for Free Radical Biology and Medicine Conference in 2017* Baltimore MD. At this meeting both were exposed to recent research advances in Redox Biology and Medicine, including a

special plenary session on mitochondria. Dr. Dong Hui Shin attended several Professional Development sessions, including grant writing and networking workshops at this meeting.

- The PI, Dr. Nadine Hempel, attended the *NIH special conference on Cancer Imaging in May 2018* in Bethesda MD. At this meeting Dr. Hempel was exposed to recent advances in imaging techniques for in vitro and in vivo imaging specific to tumor biology, with emphasis on imaging of cellular organelles.
- Dr. Dong-Hui Shin has acquired a wide new skill set in mitochondrial biology and ovarian cancer through technical training during this award period. For example he has been trained on the use of transition electron microscopy (TEM) and protein purification techniques for preparation and analysis of Drp1 variants by mass spec. He has been regularly meeting with Dr. Hempel, who has mentored Dr. Shin throughout the grant period. Dr. Shin has also benefitted from mentorship of Dr. H.G. Wang, a collaborator on the project, and has taken advantage of career development seminars through the College of Medicine, Penn State Hershey.

How were the results disseminated to communities of interest?

- Dr. Nadine Hempel delivered the following **invited research talks**:
  - "Altered Mitochondrial Form and Function in Ovarian Cancer" *University of North Carolina - Department of Cell Biology & Physiology* - Chapel Hill NC (Aug 2018)
  - "Altered Mitochondrial Form and Function in Ovarian Cancer" University of South Carolina, Department of Chemistry and Biochemistry, Colombia SC (Jan 2018)
- Dr. Donghui Shin delivered the following **invited research talks**:
  - "Mitochondrial function is dynamically regulated during ovarian cancer progression through alterations in fusion/fission" *Women's Health Research Day (WHRD). The Pennsylvania State University, University Park, PA.* (Sep 2017).
  - "The role of mitochondria fission protein Drp1 in metastatic ovarian cancer". *Annual Meeting of the Society for Redox Biology and Medicine* in Baltimore MD (Nov 2017).
- **Posters** were presented at the following meetings to disseminate our recent findings from this project (see abstracts below):
  - NIH/NHLBI special conference on mitochondria, May 2016, Bethesda MD
  - Marsha Rivkin & AACR symposium on Ovarian cancer, September 2016, Seattle WA
  - Penn State Cancer institute annual symposium, August 2017, Hershey PA

What do you plan to do during the next reporting period to accomplish the goals?
Nothing to report.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
For the first time, we have identified and verified the existence of alternate Drp1 transcript variants in ovarian cancer, and established that these have consequences on mitochondrial fission and fusion and cellular behavior and chemoresistance of ovarian cancer cells. In addition, new data emanating from this work has opened new research avenues for the investigation of Drp1 regulation during different stages of ovarian cancer metastasis.

What was the impact on other disciplines?
The role of mitochondrial dynamics has far reaching consequences beyond the field of ovarian cancer, and the basic mechanisms of mitochondrial dynamics unraveled in our work have the potential to impact pathologies where mitochondrial dysfunction is a known driver of disease, including neurodegenerative diseases.

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS

Changes in approach and reasons for change

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals.

Nothing to Report (N/A)

Significant changes in use of biohazards and/or select agents

Nothing to Report (N/A)

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications.

  - Hempel N, Trebak M. Crosstalk between calcium and reactive oxygen species signaling in cancer. *Cell calcium*. (2017) Jan 18 (in press), DoD funding acknowledged: yes

  Books or other non-periodical, one-time publications.

  Nothing to Report

  Other publications, conference papers, and presentations.

  - Shin DH, Kim YS, Shimko S, Dier U, Kesterson J, Phaeton R, Hempel N (2017) The role of mitochondria fission protein Drp1 in metastatic ovarian cancer. *Society for Redox Biology and Medicine (SfRBM) Conference, Baltimore MD*. Oral Presentation. Abstract chosen for travel award to DHS.
  - Shin DH, Kim YS, Shimko S, Wang HG, Phaeton R, Hempel N. (2017) Mitochondrial function is dynamically regulated during ovarian cancer progression through alterations in fusion/fission. *Women's Health Research Day (WHRD). The Pennsylvania State University, University Park, PA*. Oral Presentation.
  - Shin DH, Dier U, Timmins PF, Kesterson J, Phaeton R, Hempel N Mitochondrial dynamics and dysfunction in ovarian cancer. September 2016, *Rivkin Center for Ovarian Cancer and AACR – Ovarian Cancer Research Symposium, Seattle WA*. Poster Presentation.
  - Shin DH, Kim Yeon Soo, Dier Usawadee, Timmins PF, Yoon Yisang, Kesterson Joshua, Phaeton Rebecca and Hempel N. The role of mitochondria fission protein Drp1 in metastatic ovarian cancer. May 2016 *The NHLBI/NIDDK Mitochondrial Biology Symposium, National Institutes of Health, Bethesda, Maryland*. Poster Presentation.

- **Website(s) or other Internet site(s)**

  Nothing to Report

- **Technologies or techniques**

  Nothing to Report

- **Inventions, patent applications, and/or licenses**

  Nothing to Report

- **Other Products**

  Awards by Trainees:

  - 05/2016: Dong Hui Shin (Post Doc): Selected Peer Science Award, Poster presentation, The 2016 National Institutes of Health (NIH), NHLBI/NIDDK Mitochondrial Biology Symposium, Bethesda, MD
  - 10/2017: Dong Hui Shin (Post Doc): received Travel award ($500) from the Society for Redox Biology and Medicine to attend SfRBM Annual Meeting Nov 2017, Baltimore, MD
  - 06/2017: Dong Hui Shin (Post Doc): Outstanding Postdoctoral Scholar Award, Penn State College of Medicine

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Nadine Hempel
Project Role:	P.I.
Researcher Identifier:	orcid.org/0000-0002-5574-8783
Nearest person month worked:	1
Contribution to Project:	**From Budget Justification:** 5% salary is requested for the PI. Dr. Hempel will oversee the intellectual aspect and conceptual designs of the project, including experimental design, data analysis, manuscript preparation, meeting presentations and providing guidance to the research team.
Name:	Dong Hui Shin
Project Role:	Postdoctoral Scholar
Researcher Identifier:	
Nearest person month worked:	11
Contribution to Project:	**From Budget Justification: TBD Postdoctoral Scholar** (12 calendar months) 100% salary is requested for a Postdoctoral Scholar to carry out the majority of research experiments proposed, including studies pertaining to the identification of Drp1 splice forms, metabolic profiling, cell culture assays, handling of ascites derived tumor cells and mitochondrial functional studies.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Yes. Drs. Hempel, Kesterson, Phaeton, Wang, and Warrick all had changes in their active other support. Please see below.

OTHER SUPPORT - Hempel, Nadine

ACTIVE PROJECTS – Previously Reported:
None

PROJECTS FUNDED SINCE LAST REPORT:
Equipment only
(Hempel, Nadine)
1/2/2018-12/31/2018 00.00 calendar mths
Agilent Technologies DC/Yr
Contracting/Grants Officer:
Title: XFp equipment grant
Goals: Specific Aims:1) verify the clinical existence of distinct metabolic subtypes of ovarian cancer, using mitochondrial stress, fuel flex, and glycolysis stress tests; and 2) to determine if the observed ovarian cancer bioenergetics phenotypes are a valuable prognostic tool for disease outcome and susceptibility to metabolic pathway inhibitors.
Role: Principal Investigator
Overlap: N/A

(Hempel, Nadine)
8/1/2018-7/31/2019 00.12 calendar mths
Penn State Hershey Cancer Institute DC/Yr
Contracting/Grants Officer:
Title: The Mitochondrial Stress Response During Ovarian Cancer Metastasis
Goals: The hypothesis that up-regulation of the mitochondrial stress response proteins Sod2 and SIRT3 is a necessary adaptation for ovarian cancer anchorage-independent survival and metastasis is a major step in our long-term goal of identifying key stress adaptations of ovarian cancer cells that can be targeted therapeutically.
Specific Aims:AIM 1: Define the regulation of Sod2 by SIRT3 under anchorage-independent conditions, in the context of ascites derived stress signals. AIM 2: Determine the function of Sod2 as a mediator of H2O2 pro-survival signaling during transcoelomic metastasis. AIM 3: Target cells with high Sod2 status for therapeutic intervention.
Role: Principal Investigator
Overlap: none

PROJECTS ENDED SINCE LAST REPORT:
W81XWH-16-1-0117 (Hempel) 05/01/2016 – 04/30/2018 0.12 Cal Months
Department of Defense (CDMRP)
U.S. Army Medical Research and Development Command
Project Title: *Clinical Significance and Mechanistic Insights into Ovarian Cancer Mitochondrial Dysfunction*

Goals: The goal of our study is to identify the clinical relevance and mechanism leading to mitochondrial dysfunction in ovarian cancer, and identify how this influences chemo resistance and ovarian cancer etiology.

Specific Aims: 1) To establish clinical significance of mitochondrial dysfunction in EOC. 2) To elucidate the mechanistic consequences of Drp1 splice variant expression on ovarian cancer mitochondrial function, metabolism and chemo resistance. Aim 3: To investigate alternate therapeutic strategies for chemo resistant mitochondria-deficient EOCs.

Role: Principal Investigator

Overlap: *This is the project for which the progress report is being submitted.*

(Hempel, Nadine)

9/12/2010-3/31/2016 00.60 calendar mths

National Cancer Institute DC/Yr

Contracting/Grants Officer:

Title: Mitochondrial Redox Control of Metastasis

Goals: The purpose of this study was to investigate the role of sub-lethal mitochondrial ROS changes during bladder cancer metastasis and delineated their mechanisms of action as second messengers in pro-metastatic signaling events.

Specific Aims: Aim 1: To investigate the cellular site of action of mitochondrial ROS and their role in regulating oxidation, spatial distribution and protein fate of Protein Tyrosine Phosphatases (PTPs) involved in migratory signaling. Aim 2: To further delineate the redox-regulation of p130cas. Aim 3: To assess the consequences of enhanced intracellular ROS levels in an in vivo model of metastatic bladder cancer and the effectiveness of antioxidant therapy in this model.

Role: Principal Investigator

Overlap: none

(Hempel, Nadine)

4/1/2017-3/31/2018 00.60 calendar mths

Marsha Rivkin Center for Ovarian Cancer Research DC/Yr Contracting/Grants Officer: Kiran Dhilloru at (206) 215-2964 or Kiran.Dhillon@swedish.org

Title: Pre-clinical Investigation of High Dose Ascorbate IP Therapy

Goals: This study tests the feasibility of high dose ascorbate IP therapy and interrogates the molecular mechanisms of action in relationship to iron and redox stress of the ascites.

Specific Aims: This project investigates the unexplored use of high dose ascorbate as an adjuvant to intraperitoneal (IP) chemotherapy, a specific delivery strategy used to target ovarian cancer.

Role: Principal Investigator

Overlap: none

OTHER SUPPORT - Kesterson, Joshua P.

ACTIVE – PREVIOUSLY REPORTED

No Grant Number (Kesterson) August 2014 – Present 0 Cal Mos/ Unfunded Effort
Veterans of Foreign Wars of the US Ladies Auxiliary Dept. of PA Research Grant for Ovarian Cancer Research
Contracting/ Grants Officer : Brenda M. Johnson, US Ladies Auxiliary Dept of PA, 4002 Fenton Avenue Harrisburg, PA 17109
Title: *Epithelial Ovarian Cancer Research*
Goals: To be able to continue promising and exciting research endeavors in ovarian cancer research. In the absence of a sensitive screening strategy to diagnose Epithelial Ovarian Cancer (EOC) at an early stage, when survival is improved, efforts must be focused on developing new strategies to augment adjuvant cytotoxic chemotherapy to increase the interval between original diagnosis and recurrence.
Specific Aims: Aim 1: Understanding pathways by which ovarian cancer becomes resistant to standard chemotherapy
Aim 2: Means to target the OGF/OGFr axis to be susceptible to receptor agonist intervention with resultant inhibition of cell proliferation, angiogenesis and tumor growth
Role: Principal Investigator
Overlap: None

PROJECTS ENDED SINCE LAST REPORT

W81XWH-16-1-0117 (Hempel) 05/01/2016 – 04/30/2018 0.12 Cal Months
Department of Defense (CDMRP)
Contracting/ Grants Officer: U.S. Army Medical Research and Development Command Detrick, MD, 21702-5014; Josh Disbennett, Grants Management Specialist
Title: *Clinical Significance and Mechanistic Insights into Ovarian Cancer Mitochondrial Dysfunction*
Goals: The goal of our study is to identify the clinical relevance and mechanism leading to mitochondrial dysfunction in ovarian cancer, and identify how this influences chemoresistance and ovarian cancer etiology.
Specific Aims: Aim 1: To establish clinical significance of mitochondrial dysfunction in EOC.
Aim 2: To elucidate the mechanistic consequences of Drp1 splice variant expression on ovarian cancer mitochondrial function, metabolism and chemoresistance.

Aim 3: To investigate alternate therapeutic strategies for chemoresistant mitochondria-deficient EOCs.

Role: Co-Investigator

Overlap: *This is the project for which the progress report is being submitted.*

OTHER SUPPORT - Phaeton, Rebecca

ACTIVE PROJECTS PREVIOUSLY REPORTED

Grant 193394
Foundation for Women's Cancer
Genetech Gynecologic Cancer (Phaeton) 04/01/2017 to 03/31/2019 0.24 Cal Months
Young Investigator Career Development Award
Contracting/Grants Officer: Jennifer OCampo; 230 W Monroe St, Suite 710; Chicago, IL 60606-4902; phone: 312-578-1439; Jennifer.ocampo@sgo.org

Project Title: Mechanisms of ion Channels Involvement in Antibody Therapy of HPV Positive Cancers

Goals: We hypothesize that this treatment modality will 1) down-regulate volume sensitive chloride channels of the cervical epithelial membrane and 2) reverse the negative effect on CRAC channel activation in antitumor T-cells.

Specific Aims: 1) Elucidate the mechanism of entry of mAbs through cervical epithelial cell membrane and quantify mechanisms of induced immunogenicity. 2) Evaluate the changes in the cervical epithelial membrane; both functional and CI currents changes of VRACs induced by as a direct effect of mAb therapy decreasing #6 and #7 oncoprotein expression. 3) Determine CRAC channel expression, function and regulation on T-lymphocytes after mAb therapy against HPV.

Role: Principal Investigators

Overlap: None

ObGyn Departmental (Phaeton) 10/01/2016 to 09/30/2019 0.12 Cal Months
Internal Request for Application

Project Title: Development of a Biomarker for Detection of HPV Related Cervical Cancer

Goals: This proposal seeks to further expand the current knowledge by showing a relationship between specific high-risk HPV genotype and miRNA expression signatures to define new biomarkers for cancer diagnosis, treatment, and to prognosticate recurrence patterns

Specific Aims: To test the hypothesis that circulating miRNAs exhibit a differential expression signature in response to concurrent chemoradiotherapy across a spectrum of patient samples.

Role: Principal Investigator

Overlap: None

PROJECTS FUNDED SINCE LAST REPORT

Obstetrics and Gynecology (Phaeton) 10/01/2017 – 09/30/2019 0.12 Cal Months
Internal Departmental Funding

Project Title: Novel Immunotherapy Against HPV-16/18 Oncoproteins to Inhibit Cervical Cancer

Goals: This proposal will help to discover the mechanisms behind this novel viral specific therapy against cervical cancer.

Specific Aims: 1) To determine the mechanism of tumor growth inhibition in CasKi cells electroporated with C1P5 and TVG701Y: Hypothesis- C1P5 and TVG71Y induce apoptosis in tumor cells by downregulating the expression of E6 & E7 and restoration of p53 & retinoblastoma. 2) Evaluating the mechanism of mAbs mediated immune activation by co-culture: Hypothesis- C1P5 and TVG701Y interact directly with intracellular antigen and activate an immune response.

Role: Principal Investigator

Overlap: None

Extramural Loan Repayment Program 09/01/2017 – 12/31/2019 % Effort N/A
for Clinical Researchers (LRP-CR)
National Institutes of Health

Goals: These funds are a set of programs established by Congress and designed to recruit and retain highly qualified health professionals into biomedical or bio behavioral research careers. The NIH qualified health professional who contractually agree to engage in NIH mission-relevant research for at least two years initially, and who agree to engage in such research for an average of at least 20 hours per week based on a 40 hr. work week.

PROJECTS ENDED SINCE LAST REPORT

W81XWH-16-1-0117 (Hempel) 05/01/2016 – 04/30/2018 0.12 Cal Months
Department of Defense (CDMRP)
U.S. Army Medical Research and Development Command

Project Title: *Clinical Significance and Mechanistic Insights into Ovarian Cancer Mitochondrial Dysfunction*

Goals: The goal of our study is to identify the clinical relevance and mechanism leading to mitochondrial dysfunction in ovarian cancer, and identify how this influences chemo resistance and ovarian cancer etiology.

Specific Aims: 1) To establish clinical significance of mitochondrial dysfunction in EOC. 2) To elucidate the mechanistic consequences of Drp1 splice variant expression on ovarian cancer mitochondrial function, metabolism and chemo resistance. Aim 3: To investigate alternate therapeutic strategies for chemo resistant mitochondria-deficient EOCs.

Role: C*o-Investigator*

Overlap: *This is the project for which the progress report is being submitted.*

Marcia Rivkin Center for Ovarian Research (Hempel) 04/01/2017-03/31/2018 0.24 Cal Months

Project Title: *Pre-clinical Investigation of High Dose Ascorbate IP Therapy*

Goals: This project investigates the unexplored use of high dose ascorbate as an adjuvant to intraperitoneal (IP) chemotherapy, a specific delivery strategy used to target ovarian cancer. We hypothesize that the unique biochemical make-up of ascites further enhances the efficacy of IP high-dose ascorbate adjuvant therapy.

Role: Co-Investigator

Overlap: None

WU-17-231

Reproductive Scientist Development Program (Phaeton) 07/01/2014– 06/30/2017 0 Cal Months

March of Dimes / Supplemental Funding

Contracting/ Grants Officer: Amanda Heflin, Washington University School of Medicine, St. Louis

Project Title: Radioimmunotherapy: Targeting E6 and E7 viral antigens of Human Papillomavirus Induced Cervical Cancer

Goals: The additional funding from this supplemental grant will be used to perform the studies of mice treated with 177 Lu labeled antibodies with assessment of toxicities of white blood cells, platelets, liver function tests and long term assessment of bone marrow evaluation.

Specific Aims: To assess dose limiting toxicities of treatment in vital organs and establishing iodistribution patters of most effective radiolanthanide noted in Aim 1 in our RSDP Seed Grant.

Role: Principal Investigator

Overlap: None

Association of Faculty and Friends Translational Ovarian Cancer Research (Phaeton) 07/01/2016 – 06/30/2017 Unfunded Effort

Project Title: None

Goals: Funding is to support and promote medical education, research, clinical care and scholarships

Specific Aims: Translational Ovarian Cancer Research. This funding is designated specifically for Capital Equipment for the incubator and consumables and cell culture reagents.

Role: Principal Investigator

Overlap: None

OTHER SUPPORT - Wang, Hong-Gang

ACTIVE-Previously Reported

P01 CA171983-05 Project 1 (Wang, Hong-Gang) 9/10/2013-8/31/2018 0.36 calendar

NIH/NCI prime, Subaward from University of Virginia Contracting/Grants Officer: Robert Wilson, Cancer Center Senior Finance Generalist, UVA Cancer Center, Rw9u@hscmail.mcc.virginia.edu
Title: "Targeted Sphingolipid Metabolism for Treatment of AML" (Kester/Loughran/Wang)
The Program's broad-long-term objective is to develop new targeted therapeutics for acute myelogenous leukemia (AML).
Specific Aims: Aim 1: Engineer, characterize and optimize novel lipomimetic- or small molecule-based therapeutics for AML
Aim 2: Validate the efficacy and toxicology of sphingolipid-targeted therapeutics in vivo using murine leukemia stem cells models.
Aim 3: Define the role of altered sphingofipid metabolism in cell survival, apoptosis, autophagy, and drug resistance in AML.
Project 1: "Targeting Ceramide Metabolism in AML" (Kester, Mark)
The overall goal of Project 1 is the design of second-generation ceramide nanoliposomes that exert efficacy in AML patients who are resistant to conventional chemotherapy.
Role: Site PI
Overlap: None

P01 CA171983-05 Core C (Claxton, David F.) 9/10/2013-8/31/2018 0.36 calendar
NIH/NCI prime, Subaward from University of Virginia
(3% salary support only)
Contracting/Grants Officer: Robert Wilson, Cancer Center Senior Finance Generalist, UVA Cancer Center, Rw9u@hscmail.mcc.virginia.edu
Title: "Targeted Sphingolipid Metabolism for Treatment of AML" (Kester/Loughran/Wang)
The Program's broad-long-term objective is to develop new targeted therapeutics for acute myelogenous leukemia (AML).
Specific Aims: Aim 1: Engineer, characterize and optimize novel lipomimetic- or small molecule-based therapeutics for AML
Aim 2: Validate the efficacy and toxicology of sphingolipid-targeted therapeutics in vivo using murine leukemia stem cells models.
Aim 3: Define the role of altered sphingofipid metabolism in cell survival, apoptosis, autophagy, and drug resistance in AML.
Core C: "Animal Modeling and Clinical Resources Core" (Claxton, David)
This Core will serve an essential role for all projects by maintaining a repository of human AML samples with defined clinical outcomes, as well as by developing robust murine models to test the efficacy of the project's sphingolipid-based AML therapeutics.
Role: Co-Investigator
Overlap: None

P01 CA171983-05 Proj 3 (Wang, Hong-Gang) 9/10/2013-8/31/2018 1.20 calendar
NIH/NCI prime, Subaward from University of Virginia
Contracting/Grants Officer: Robert Wilson, Cancer Center Senior Finance Generalist, UVA Cancer Center, Rw9u@hscmail.mcc.virginia.edu
Title: "Targeted Sphingolipid Metabolism for Treatment of AML" (Kester/Loughran/Wang)

The Program's broad-long-term objective is to develop new targeted therapeutics for acute myelogenous leukemia (AML).
Specific Aims: Aim 1: Engineer, characterize and optimize novel lipomimetic- or small molecule-based therapeutics for AML
Aim 2: Validate the efficacy and toxicology of sphingolipid-targeted therapeutics in vivo using murine leukemia stem cells models.
Aim 3: Define the role of altered sphingofipid metabolism in cell survival, apoptosis, autophagy, and drug resistance in AML.
Project 3: "Targeting Sphingosine Kinase in AML" (Wang, Hong-Gang)
The long-term goal of Project 3 is to translate our potent small molecule inhibitors of sphingosine kinase 1 (SphK1) to clinical use in the treatment of acute myeloid leukemia (AML).
Role: Site PI
Overlap: None

P01 CA171983-05 Core E (Wang, Hong-Gang) 9/10/2013-8/31/2018 0.32 calendar
NIH/NCI prime, Subaward from University of Virginia
(2.68% salary support only)
Contracting/Grants Officer: Robert Wilson, Cancer Center Senior Finance Generalist, UVA Cancer Center, Rw9u@hscmail.mcc.virginia.edu
Title: "Targeted Sphingolipid Metabolism for Treatment of AML" (Kester/Loughran/Wang)
The Program's broad-long-term objective is to develop new targeted therapeutics for acute myelogenous leukemia (AML).
Specific Aims: Aim 1: Engineer, characterize and optimize novel lipomimetic- or small molecule-based therapeutics for AML
Aim 2: Validate the efficacy and toxicology of sphingolipid-targeted therapeutics in vivo using murine leukemia stem cells models.
Aim 3: Define the role of altered sphingofipid metabolism in cell survival, apoptosis, autophagy, and drug resistance in AML.
Core E: "Administrative Core" (Kester/Loughran/Wang)
The overarching goal of the Administrative Core is to oversee and coordinate all scientific, regulatory, administrative and fiscal responsibilities of the Program Project grant.
Role: Site PI
Overlap: None

5R01 GM117014-03 (Miller, Barbara A.) 5/1/2016-4/30/2020 0.24 calendar
NIH/NIGMS
(2% salary support only)
Contracting/Grants Officer: Director, Center for Scientific Review , National Institutes of Health Health, 6701 Rockledge Drive MSC 7768, Bethesda MD 20892-7768
Title: "TRPM2, Mitochondria, and Cell Survival"
Goals: This research will investigate whether TRPM2-L sustains cell proliferation and protects viability through moderate Ca2+ influx, which mediates HIF-1/2α expression, maintains mitochondrial function, and reduces production of reactive oxidant species.

Specifi Aims: Aim 1: Is cell proliferation or viability reduced by inhibition of TRPM2 mediated Ca2+ influx? We will determine the role of TRPM2-mediated Ca2+ influx in modulation of cell viability, in vivo tumor formation, doxorubicin sensitivity, and ROS generation using TRPM2 loss and gain of function mutants.
Aim 2: Does TRPM2-mediated Ca2+ influx modulate mitochondrial function in neuroblastoma? Mitochondrial function including mitochondrial membrane potential, Ca2+ uptake, ATP production, and expression of BNIP3 and NDUFA4L2 are significantly reduced in cells expressing TRPM2-S, and ROS production is increased. Role: Co-Investigator
Overlap: None

PROJECTS FUNDED SINCE LAST REPORT:

209833 (Wang, Hong-Gang, Miller, Barbara A.) 12/01/2018-11/30/2019 0.36 calendar
Hyundai Hope on Wheels
Contracting/Grants Officer: Zafar J. Brooks, (714) 965-3584, 10550 Talbert Ave Fountain Valley, CA 92708, info@HopeOnWheels.org
Title: "Exploring a Novel Therapeutic Approach for FLT3-ITD Pediatric AML"
Goals: The goals of this project are to better understand non-mutational drug resistance mechanisms and to identify novel therapeutic strategies to decrease relapse and improve long-term survival in the highest-risk population of children with FLT3-mutated AML.
Specific Aims: Aim 1: Validate if the combination of FLT3 inhibitors with anti-inflammatory drugs induces synergistic cell killing in additional FLT3-ITD AML cell lines and primary patient samples and explore the underlying mechanisms.
Aim 2: Determine if the combination of FLT3 inhibitors with anti-inflammatory drugs reduces AML relapse and improve survival in patient-derived xenograft (PDX) and syngeneic mouse models of AML in the absence and presence of a functional immune system, respectively.
Role: Co-PI
Overlap: None

1 R01 GM127954-01 (Wang, Hong-Gang) 07/01/2018-03/31/2022 3.00 calendar
NIH/NIGMS
Contracting/Grants Officer: Jennifer Billington, billingj@nigms.nih.gov, (301) 594-5243, 6701 Rockledge Drive Bethesda, MD 20892-7768
Title: "Autophagosome closure by the ESCRT machinery"
Goals: The goal of this project is to determine the mechanism by which the ESCRT machinery regulates autophagosome closure.
Specific Aims: Aim 1: Identification the core ESCRT machinery required for phagophore closure
Aim 2: Characterization the molecular signals for ESCRT recruitment to phagophores
Role: PI
Overlap: None

1 R01 CA222349-01 (Wang, Hong-Gang) 08/01/2018-07/31/2023 3.00 calendar
National Cancer Institute

Contracting/Grants Officer: Leslie Hickman, hickmanl@mail.nih.gov, (301) 631-3009, 6701 Rockledge Drive Bethesda, MD 20892-7768
Title: "Non-canonical Caspase-8 Activation on Autophagosomal Membranes"
Goals:
The goal of this project is to test the hypothesis that an accumulation of immature autophagosomal membranes induces the non-canonical activation of caspase-8 to switch cytoprotective autophagy to apoptosis
for a novel anti-cancer strategy.
Specific Aims: Aim 1: To demonstrate that impaired phagophore closure promotes iDISC-mediated caspase-8 activation and identify molecular regulators of non-canonical caspase-8 activation.
Aim 2: To test the hypothesis that ATG2A/B and VMP1 regulate phagophore closure through the delivery of ATG9-containing membranes.
Aim 3: To demonstrate that impaired phagophore closure can switch autophagy to iDISC-mediated apoptosis in vivo and suppress the progression of MLL-rearranged AML.
Role: PI
Overlap: None

PROJECTS ENDED SINCE LAST REPORT:
W81XWH-16-1-0117 (Hempel, Nadine) 5/1/2016-4/30/2018 0.12 calendar
U. S. Army Medical Research and Development Command
Contracting/Grants Officer: Joshua Disbennett, Grant Specialist, Grants Administration Office, 301-619-7349, Joshua.L.Disbennett.civ@mail.mil
Title: "Clinical Significance and Mechanistic Insights into Ovarian Cancer Mitochondrial Dysfunction"
Goals: The goal of our study is to identify the clinical relevance and mechanism leading to mitochondrial dysfunction in ovarian cancer, and identify how this influences chemoresistance and ovarian cancer etiology. Specific Aims: Aim 1: To establish clinical significance of mitochondrial dysfunction in EOC. Aim 2: To elucidate the mechanistic consequences of Drp1 splice variant expression on ovarian cancer mitochondrial function, metabolism and chemoresistance.
Aim 3: To investigate alternate therapeutic strategies for chemoresistant mitochondria-deficient EOCs.
Role: Co-Investigator
Overlap: *This is the project for which the progress report is being submitted.*

OTHER SUPPORT - Warrick, Joshua

ACTIVE PROJECTS –Previously Reported
None

PROJECT FUNDED SINCE LAST REPORT:
IRG-17-175-04 Warrick and Vonn Walter (co-PIs) 07/01/2018 – 06/30/2019 0.6 unfunded cal months
American Cancer Society – Institutional Review Grant
Grants Officer: None

Title: Social Determinants, Incidence, Outcomes, and Molecular Biology of Urinary Bladder Cancer in Central Pennsylvania
Project goal: We propose to identify biological differences in bladder cancer by socioeconomic status, as well as identify differences in bladder cancer outcomes by socioeconomic status.
Specific Aims: Specific Aim 1: To examine the association of demographic, socioeconomic, geographic, and tumor pathology (stage and grade) factors with urinary bladder cancer risk and outcomes in Pennsylvania, including the 28-county Penn State Cancer Institute (PSCI) catchment area. Sub-Aim A: Examine these factors in The Community Sciences and Health Outcomes Shared Resource, a repository of all bladder cancers diagnosed in the state of Pennsylvania. Sub-Aim B: Examine these factors in a cohort of bladder cancer patients treated at PSCI. Specific Aim 2: To identify genomic signatures that account for disparities in bladder cancer outcomes among people with differing socioeconomic status - disparities that persist when accounting for tumor grade, tumor stage, and cancer risk factors.

OVERLAP: None

No number DeGraff (PI) 07/01/2018 – 06/30/2022 0.24 cal months
American Cancer Society
Grants Officer: Michael H. Melner, PhD, ACS, 250 Williams Street, Atlanta, GA 30303, Tel #: 404-327-6528, Michael.melner@cancer.org
Title: Transcriptional Control of Bladder Cancer Progression
Project goal: we propose experiments to understand the role of TFAP2 family members in chemoresponse. Successful completion of proposed experiments may identify PPARG and/or TFAP2 family members as targets to slow tumor progression and/or markers of chemosensitivity, which would address the most pressing problems in this area of study.
Specific Aims: Specific Aim 1: *Define the regulatory and functional relationship between PPARɣ and TFAP2A*. Decreased PPARɣ expression and pathway activity, as well as increased TFAP2A expression are associated with development of a basal molecular subtype of BC. Our preliminary data unify these observations by identifying PPARɣ as a negative regulator of TFAP2A in BC cells. Therefore, we will determine if TFAP2A is a direct target of PPARɣ. As our observations additionally suggest PPARɣ inactivation promotes the basal molecular subtype by increasing TFAP2A expression, we will conduct mechanistic experiments to determine if the influence of PPARɣ on molecular subtype is dependent upon changes in TFAP2A expression.
Specific Aim 2: *Determine the ability of TFAP2A and/or TFAP2C to promote BC progression and chemoresponse in vitro* and *in vivo.* Preliminary data suggest a role for TFAP2A and TFAP2C expression in the development of clinically aggressive BC, and TFAP2C overexpression promotes tumorigenicity of T24 and UMUC3 BC cells. Therefore, we will perform *in vitro* proliferation and invasion assays, as well as novel *in vivo* tissue recombination experiments to determine the impact of altered expression of TFAP2A and TFAP2C on BC cell growth, invasion, tumorigenicity and chemosensitivity.
Specific Aim 3: *Determine the impact of Tfap2a and Tfap2c knockout on carcinogen-induced basal BC and response to cisplatin.* The BBN model of bladder carcinogenesis is an excellent and accepted model system for the study of basal BC, as exposure of mice to this bladder-specific carcinogen results in advanced disease with extensive SqD complete with a basal gene expression pattern (7). Therefore,

we will use an inducible Krt5-driven Cre system to test the impact of *Tfap2a and Tfap2c* knockout (KO) within the tumor cell of origin on the development of basal BC and chemosensitivity.

OVERLAP: None

PROJECTS ENDED SINCE LAST REPORT:

(Warrick, Joshua, PI) 1/1/2016-12/31/2017 0 cal mths
PA Tobacco Settlement Fund (TSF)
DC
Contracting/Grants Officer: John Anthony, Coordinator, Research and Administrative Services, Penn State University, 110 Technology Center, University Park, PA, 16802, jta11@psu.edu
Title: Cancer Risk Stratification of Endometrial Hyperplasia by Next Generation Sequencing
Goals: Determine if cancer gene sequencing can distinguish between benign and premalignant endometrial hyperplasia.
Specific Aims: Aim 1: Perform a case-control study to determine if extended mutational analysis of endometrial hyperplasia is predictive of cancer risk. Aim 2: establish the mutational landscape of endometrial hyperplasia.
Role: Principal investigator

OVERLAP: None
W81XWH-16-1-0117 Hempel (PI) 04/01/2016 - 03/31/2018 0.12 cal months
Department of Defense, Ovarian Cancer Pilot Award
D.C.
Grants Officer: Elena Howell, 1077 Patchel Street, Fort Dietrick, MD 21702, 301-619-6871
Title: Clinical Significance and Mechanistic Insights into Mitochondrial Dysfunction of Ovarian Cancer
Project goal: In the long term, it is our hope that the knowledge gained from the present work can be adapted to the clinic for the identification and treatment of this unique group of highly chemoresistant ovarian cancers.
Specific Aims: Aim 1: To establish clinical significance of mitochondrial dysfunction in EOC. In a cohort of ovarian cancer patients, we will: 1A: Test if mitochondrial dysfunction is a common clinical phenotype of highly chemoresistant EOCs. 1B: Determine the identity and frequency of expression of Drp1 splice variants, and examine their association with mitochondrial dysfunction and chemoresistance. 1C: Test if DNM1L copy number alterations correlated with Drp1 expression. Aim 2: To elucidate the mechanistic consequences of Drp1 splice variant expression on EOC mitochondrial function, metabolism, and chemoresistance. 2A: Test if identified Drp1 transcript variants act as dominant negative fission proteins. 2B: Determine if Drp1-mediated mitochondrial dysfunction aids in chemoresistance by disrupting programmed cell death. 2C: Assess if Drp1-mediated mitochondrial dysfunction contributes to chemoresistance by eliciting cellular DNA damage response.
Aim 3: To investigate alternate therapeutic strategies for chemoresistant mitochondria-deficient EOCs. 3A: Determine the alternate metabolic pathways used by mitochondria-defective cells. 3B:

Assess if disruption of alternate metabolic pathways by metabolism-based inhibitors represents a novel therapeutic strategy to target mitochondria-defective cells.

OVERLAP: *This is the project for which the final report is being submitted.*

What other organizations were involved as partners?

Nothing to Report (N/A)

8. SPECIAL REPORTING REQUIREMENTS

N/A

COLLABORATIVE AWARDS:

N/A

QUAD CHARTS:

N/A

9. APPENDICES:

- Abstracts and Posters:
    - Shin DH, Kim YS, Shimko S, Dier U, Kesterson J, Phaeton R, Hempel N (2017) The role of mitochondria fission protein Drp1 in metastatic ovarian cancer. *Society for Redox Biology and Medicine (SfRBM) Conference, Baltimore MD*.
    - Shin DH, Dier U, Timmins PF, Kesterson J, Phaeton R, Hempel N Mitochondrial dynamics and dysfunction in ovarian cancer. September 2016, Rivkin Center for Ovarian Cancer and AACR – Ovarian Cancer Research Symposium, Seattle WA.
    - Shin DH, Kim Yeon Soo, Dier Usawadee, Timmins PF, Yoon Yisang, Kesterson Joshua, Phaeton Rebecca and Hempel N. The role of mitochondria fission protein Drp1 in metastatic ovarian cancer. May 2016 The NHLBI/NIDDK Mitochondrial Biology Symposium, National Institutes of Health, Bethesda, Maryland.

SFRBM 2017 Baltimore MD

Mitochondrial function is dynamically regulated during ovarian cancer progression through alterations in fusion/fission.

Dong-Hui Shin[1], Yeon Soo Kim[1], Sara Shimko[1], Hong-Gang Wang[2], Rebecca Phaeton[3], and Nadine Hempel[1]
[1]Department of Pharmacology, Pennsylvania State University College of Medicine, Hershey, PA, USA
[2]Department of Pediatrics, Pennsylvania State University College of Medicine, Hershey, PA, USA
[3]Department of Gynecology and Obstetrics, Pennsylvania State University College of Medicine, Hershey, PA, USA

Abstract (1900 characters)

Ovarian cancer continues to be the most deadly gynecological malignancy. A large proportion of patients are diagnosed at late stage, when significant metastasis has occurred through the intraperitoneal (IP) cavity. Preliminary work suggests that changes in mitochondrial function play important roles in tumor growth, metastasis and chemoresistance, and that this can be dynamically regulated dependent on tumor stage. Our data suggest that under proliferative attached conditions the high-grade serous adenocarcinoma cell line OVCA420 can shut-down mitochondrial function by altering its fission/fusion dynamics. Significant hyperfusion is associated with increased reliance on glycolysis and specific expression patterns of Drp1 splice variants, the GTPase responsible for mitochondrial fission. Of clinical relevance, a subgroup of cancer cells derived from patients diagnosed with late stage disease show similar patterns of Drp1 expression and mitochondria dysfunction. Characterization of these Drp1 splice variants is underway to determine their role as dominant negative fission proteins in ovarian cancer. Interestingly, we find that expression of Drp1 variants is dynamically regulated during different stages of ovarian cancer metastasis. Expression of Drp1 variants with apparent dominant negative function are decreased when cells are cultured as cellular spheroid aggregates, which models anchorage-independent survival and cells detaching from the primary tumor into the IP cavity during transcoelomic spread. This is accompanied by restoration of mitochondrial fission/fusion dynamics and increased auto/mitophagy, suggesting that cancer cells in anchorage-independent conditions alter their fission protein expression to maintain a healthy pool of mitochondria, while expression of dominant negative Drp1 is associated with a highly proliferative cellular phenotype. In conclusion, dynamic Drp1 expression switching between the dominant-negative and functional Drp1 fission proteins may be a novel way for cancer cells to regulate fission/fusion, mitochondrial quality control and metabolism.

Mitochondrial dynamics and dysfunction in ovarian cancer

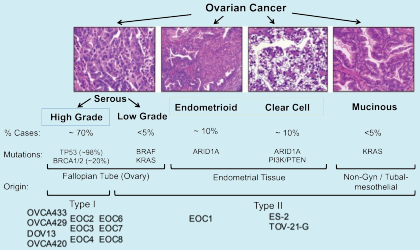
Dong-Hui Shin[1], Usawadee Dier[2], Patrick F. Timmins[3], Joshua Kesterson[4], Rébécca Phaëton[4], and Nadine Hempel[1]

[1]Department of Pharmacology, Pennsylvania State University College of Medicine, Hershey, PA, USA
[2]Nanobioscience Constellation, State University of New York Polytechnic Institute, Albany, NY, USA
[3]Women's Cancer Care Associates, Albany, NY, USA
[4]Department of Gynecology and Obstetrics, Pennsylvania State University College of Medicine, Hershey, PA, USA

PennState College of Medicine

Abstract

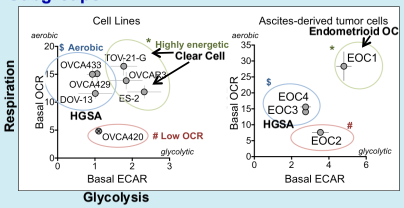
Altered mitochondrial function remains a key feature of many tumor cells and drives pathways such gene expression, metabolic and stress responses, cell cycle progression and resistance to apoptosis. Many chemotherapeutics activate programmed cell death and it is thought that mitochondrial dysfunction may be one mechanism by which cancer cells evade killing by these compounds. Screening the metabolic profiles of ovarian cancer cell lines and patient ascites-derived tumor cells reveals that ovarian cancers fall into unique bioenergetic subgroups. For example, Ovarian Clear Cell Carcinomas (OCCC) display high oxygen consumption rate and glycolytic flux compared to the more common high grade serous adenocarcinoma (HGSA) subtype. In addition, we show that a portion of HGSAs have severe mitochondrial dysfunction, that is marked by a decrease in mitochondrial respiration, a lack of response to the uncoupler FCCP and a concomitant reliance on alternate metabolic pathways. Moreover, this is accompanied by enhanced chemoresistance to Cisplatin and Taxol. The cause of mitochondrial dysfunction has been attributed to a number of factors, including deregulation in mitochondrial fission/fusion dynamics. Moreover, fission is an integral component of apoptotic and autophagy pathways. Interestingly, the observed HGSA mitochondrial dysfunction correlates with aberrant fusion/fission dynamics and expression of a low molecular weight variant of the mitochondrial fission protein Drp1. The potential significance of Drp1 in ovarian cancer etiology is highlighted by TCGA data, where more than 15% of HGSA samples display significant increases in Drp1 mRNA levels, and associated amplification of the Drp1 gene *DNM1L*. Whether this represents the shorter, potentially dominant-negative Drp1 variant identified in our work is currently under active investigation. Our data suggest that compromised mitochondrial function and fission/fusion dynamics may be a hallmark of a previously unidentified subgroup of highly chemoresistant EOCs and that this is associated with aberrant expression of the fission protein Drp1. Studies are underway to identify the molecular identity and regulation of short Drp1, and the mechanistic links to alterations in fission, metabolic switching and chemoresistance.



Ovarian Cancer histological subtypes (modified from Rescigno *et al.*, 2013). Cell lines and patient ascites-derived tumor cells (EOC) used in the study are listed below their classification. Ascites were obtained from stage III-IV ovarian cancer patients (SUNY / Penn State Hershey IRB approved study). Ovarian cancer cells were isolated and cultured, as previously described (Shepherd *et al.*, 2006 Nat Protoc;1:2643).

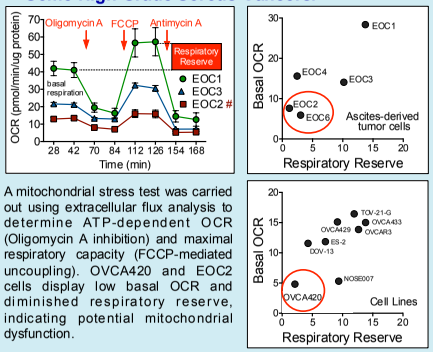
Results

1. Ovarian Cancers Fall Into Unique Bioenergetic Subgroups.



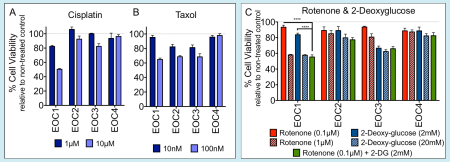
Bioenergetics profiles were obtained by assessing Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR) using a Seahorse Extracellular Flux analyzer (Dier *et al.*, 2014, PLoS ONE, 9:e98479). Ascites-derived endometrioid ovarian cancer cells (EOC1) and Clear Cell Carcinoma cell lines (TOV-21-G, ES-2) display a highly energetic phenotype (*; high glycolysis-ECAR / high respiration - OCR). OVCA420 and EOC2, both high grade serous adenocarcinomas (HGSA) had very low basal OCR, indicative of low mitochondrial respiration (#).

2. Mitochondrial Dysfunction Is A Phenotype Of Some High Grade Serous Cancers.



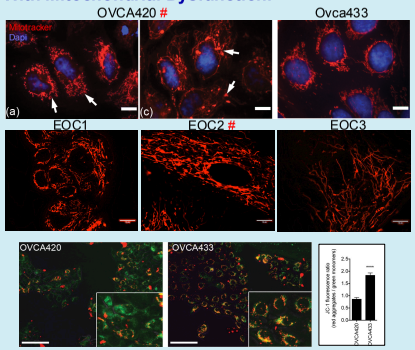
A mitochondrial stress test was carried out using extracellular flux analysis to determine ATP-dependent OCR (Oligomycin A inhibition) and maximal respiratory capacity (FCCP-mediated uncoupling). OVCA420 and EOC2 cells display low basal OCR and diminished respiratory reserve, indicating potential mitochondrial dysfunction.

3. Correlation Of Bioenergetics With Chemoresistance And Response To Metabolic Inhibitors.



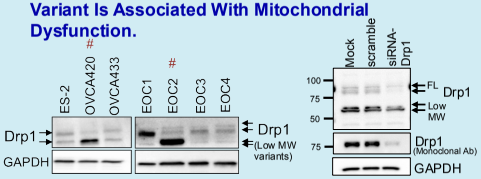
Cell viability in response to compounds was assessed using crystal violet staining, following 72 hrs of treatment. n=6, ****p<0.001 (ANOVA) Combination treatment of mitochondrial poisoning and glycolysis inhibition effectively target highly energetic ovarian cancer cells.

4. Aberrant Mitochondrial Fission/Fusion Correlates With Mitochondrial Dysfunction.



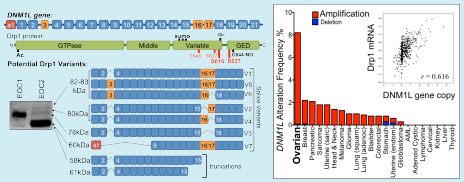
Mitotracker CMX ROS staining was used to visualize mitochondrial morphology. Arrows show areas of irregular mitochondrial morphology and large globular aggregates in OVCA420 and EOC2 cells (#). OVCA420 cells display a loss of mitochondrial membrane potential (JC-1 dye).

5. Expression Of A Low Molecular Weight Drp1 Variant Is Associated With Mitochondrial Dysfunction.



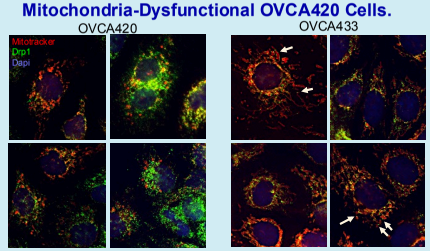
Cells with irregular mitochondrial fission (OVCA420 & EOC2 #) display enhanced expression of a low MW (~55-60kDa) variant of the fission protein Drp1. Transfection of an siRNA smartPool confirms that the observed low MW variant is Drp1.

6. Potential *DNM1L*/Drp1 Variants And *DNM1L* Copy Number Variation / Expression In Ovarian Cancer.



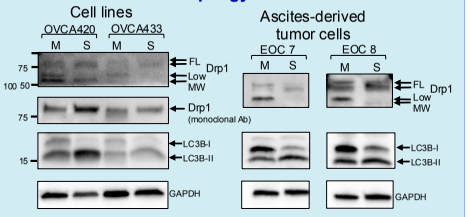
The *DNM1L* gene encodes for Drp1. Splice variants depicted are based on sequences from GenBank. RNA sequencing and mass spec analysis are underway to identify the observed variants in EOC samples and Ovarian cancer cell lines. Interestingly, Ovarian Cancer has a high frequency of *DNM1L* gene amplifications compared to other cancers, and expression of Drp1 correlates with *DNM1L* copy number (log2; TCGA derived data, CBioportal). We are investigating if certain splice and truncation variants represent dominant negative forms of Drp1 in ovarian cancer.

7. Drp1 Is Miss-localized To The Cytoplasm In Mitochondria-Dysfunctional OVCA420 Cells.



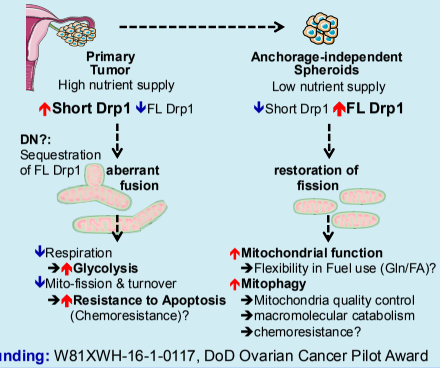
Immunofluorescence staining of Drp1 reveals distinct punctate localization at mitochondrial fission sites in OVCA433 cells (arrows), while Drp1 is distributed in the cytosol in OVCA420 cells.

8. Expression Of Low MW And Full Length Drp1 Is Dynamic Depending On Culture Conditions And Correlates With Autophagy.



Cells grown as anchorage-independent spheroids loose expression of low MW Drp1 and increase expression of full length Drp1. This switch is associated with increased LC3B-II levels, and indication of increased auto/mitophagy in spheroids.

Model



Funding: W81XWH-16-1-0117, DoD Ovarian Cancer Pilot Award

Rivkin & AACR Ovarian Cancer research Symposium, September 2016, Seattle WA

Abstract:

Mitochondrial dynamics and dysfunction in ovarian cancer

Dong-Hui Shin[1], Usawadee Dier[2], Patrick F. Timmins[3], Joshua Kesterson[4], Rebecca Phaeton[4] and Nadine Hempel[1*]

[1]Department of Pharmacology, Pennsylvania State University College of Medicine, Hershey, PA, USA
[2]Nanobioscience Constellation, State University of New York Polytechnic Institute, Albany, NY, USA
[3]Women's Cancer Care Associates, Albany, NY, USA
[4]Department of Gynecology and Obstetrics, Pennsylvania State University College of Medicine, Hershey, PA, USA

* Presenting Author

Altered mitochondrial function remains a key feature of many tumor cells and drives pathways such gene expression, metabolic and stress responses, cell cycle progression and resistance to apoptosis. Many chemotherapeutics activate programmed cell death and it is thought that mitochondrial dysfunction may be one mechanism by which cancer cells evade killing by these compounds. Screening the metabolic profiles of ovarian cancer cell lines and patient ascites-derived tumor cells reveals that ovarian cancers fall into unique bioenergetic subgroups. For example, Ovarian Clear Cell Carcinomas (OCCC) display high oxygen consumption rate and glycolytic flux compared to the more common high grade serous adenocarcinoma (HGSA) subtype. In addition, we show that a portion of HGSAs have severe mitochondrial dysfunction, that is marked by a decrease in mitochondrial respiration, a lack of response to the uncoupler FCCP and a concomitant reliance on alternate metabolic pathways. Moreover, this is accompanied by enhanced chemoresistance to Cisplatin and Taxol. The cause of mitochondrial dysfunction has been attributed to a number of factors, including deregulation in mitochondrial fission/fusion dynamics. Moreover, fission is an integral component of apoptotic and authophagy pathways. Interestingly, the observed HGSA mitochondrial dysfunction correlates with aberrant fusion/fission dynamics and expression of a low molecular weight variant of the mitochondrial fission protein Drp1. The potential significance of Drp1 in ovarian cancer etiology is highlighted by TCGA data, where more than 15% of HGSA samples display significant increases in Drp1 mRNA levels, and associated amplification of the Drp1 gene *DNM1L*. Whether this represents the shorter, potentially dominant-negative Drp1 variant identified in our work is currently under active investigation. Our data suggest that compromised mitochondrial function and fission/fusion dynamics may be a hallmark of a previously unidentified subgroup of highly chemoresistant EOCs and that this is associated with aberrant expression of the fission protein Drp1. Studies are underway to identify the molecular identity and regulation of short Drp1, and the mechanistic links to alterations in fission, metabolic switching and chemoresistance.

NHLBI Symposium on Mitochondria, May 2016, Bethesda, MD

Abstract:

The role of mitochondria fission protein Drp1 in metastatic ovarian cancer

Dong-Hui Shin[1], Yeon Soo Kim[1], Usawadee Dier[2], Patrick F. Timmins[3], Yisang Yoon[4], Joshua Kesterson[5], Rebecca Phaeton[5] and Nadine Hempel[1]

[1]Department of Pharmacology, Pennsylvania State University College of Medicine, Hershey, PA, USA
[2]Nanobioscience Constellation, State University of New York Polytechnic Institute, Albany, NY, USA
[3]Women's Cancer Care Associates, Albany, NY, USA
[4]Department of Physiology, Medical College of Georgia, Georgia Regents University, Augusta, GA, USA
[5]Department of Gynecology and Obstetrics, Pennsylvania State University College of Medicine, Hershey, PA, USA

Background Ovarian cancer remains the most deadly gynecological malignancy. A large proportion of patients are diagnosed at late stage, when significant metastasis has occurred through the intra peritoneal (IP) cavity and this is characterized by high rates of relapse and chemoresistance. Mitochondrial dysfunction plays an important role in both tumor growth, metastasis and chemoresistance. On the contrary, functional mitochondria are integral to proper apoptosis induction and it is thought that mitochondrial dysfunction is one mechanism by which cancer cells evade killing by this pathway. We recently showed that the ovarian high-grade serous adenocarcinoma cell line OVCA420 has dysfunctional mitochondria. In addition, a subgroup of cancer cells derived from ascites fluid of patients diagnosed with late stage disease showed a similar phenotype. The cause of mitochondrial dysfunction has been attributed to a number of factors including deregulation in mitochondrial fission and fusion dynamics. Here we investigated the role of different Drp1 variants in regulation of mitochondrial dynamics and function in ovarian cancer.

Methods Mitochondrial function of ovarian cancer cell lines and cells derived from ovarian cancer patients ascites fluid was assessed using Seahorse extracellular flux analysis. Mitotracker staining was used to analyze mitochondria morphology and expression levels of mitochondria fission related protein Drp1 determined using immunoblotting. The gene expression levels of cancer stem cell (CSC) markers in OVCA 420 cellular spheroid aggregates were assessed using Human Stem Cell RT2 profiler PCR Array.

Results We found that OVCA420 cells and one of 4 patient derived samples showed very low basal oxygen consumption rate and a lack in respiratory reserve capacity compared to other ovarian cancer cell lines, while relying primarily on glycolysis and glutamate utilization. Further, these cells displayed highly disordered mitochondria morphology with areas of high Mitotracker aggregation. We identified that the expression of a low molecular weight variant of the mitochondria fission protein Drp1 (short Drp1) is highly expressed in ovarian cancer with mitochondrial dysfunction. Importantly, a subgroup of cancer cells from patients also had overexpression of this short Drp1. This suggests that short Drp1 may represent a dominant negative form that inhibits mitochondrial fission, inhibiting mitochondrial function, and altering tumor metabolism. In addition, we found that expression of short and full length Drp1 expression are dynamically regulated during different stages of ovarian cancer progression. We investigated the formation of cellular spheroid aggregates, as a model of cells detaching from the primary tumor into the IP cavity. We found that short Drp1 expression is decreased and full length Drp1 protein is increased in spheroid aggregates. In addition, CSC-associated transcription factors Oct4 and Nanog were increased in spheroids. We are investigating if expression of short, potentially dominant-negative Drp1 is associated with a highly proliferative cellular phenotype, while full length active Drp1 is increased in CSC-enriched spheroids to maintain a healthy pool of mitochondria during anchorage independence.

Conclusion Dynamic Drp1 expression switching between the dominant-negative short and the full length Drp1 fission protein may be a novel way for cancer cells to regulate fission/fusion, mitochondrial quality control and metabolism.

The role of mitochondria fission protein Drp1 in metastatic ovarian cancer

Dong-Hui Shin[1], Yeon Soo Kim[1], Usawadee Dier[2], Patrick F. Timmins[3] and Nadine Hempel[1]

PENNSTATE HERSHEY College of Medicine

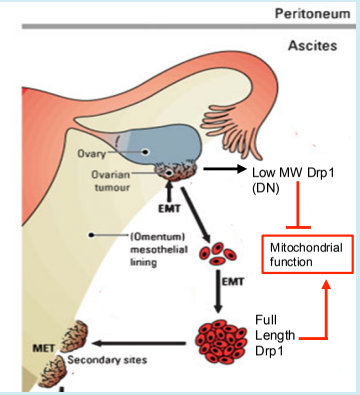
[1]Department of Pharmacology, Pennsylvania State University College of Medicine, Hershey, PA, USA
[2]Nanobioscience Constellation, State University of New York Polytechnic Institute, Albany, NY, USA
[3]Women's Cancer Care Associates, Albany Medical College, Albany NY, USA

Background

- Ovarian cancer remains the most deadly gynecological malignancy[1].
- A large proportion of patients are diagnosed at late stage, when significant metastasis has occurred through the peritoneal cavity and the disease is characterized by high rates of relapse and chemoresistance[2].
- Mitochondrial dysfunction is frequently observed in metastatic cancer cells[3], and may be related to metabolic adaptations of cancer cells, such as glycolysis.
- Functional mitochondria are integral to proper apoptosis induction and it is thought that mitochondria dysfunction may be one mechanism by which cancer cells evade killing by this pathway.
- The cause of mitochondria dysfunction has been attributed to a number of factors including deregulation in mitochondrial fission and fusion dynamics[4,5].

Hypothesis

Our hypothesis is that metastatic ovarian cancer cells dynamically regulate their mitochondrial function by alternate expression of Drp1 protein variants, depending on their stage of metastatic spread and energy demands.



Results

Bioenergetics Profiling identifies Ovarian Cancer cells with Mitochondrial Dysfunction

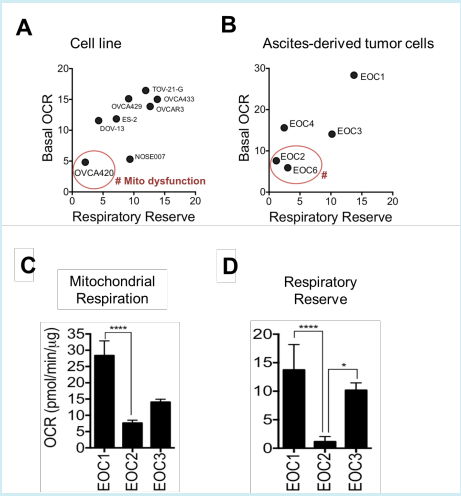


Figure 1. Extracellular flux analysis of ovarian cancer cell lines (A) and ascites-derived EOCs (B) identified a sub-set of mitochondria defective cells (#) with low oxygene consumption rate (OCR, C) and low Respiratory reserve capacity (D). *p < 0.05, ****p 0.001 (ANOVA)

Mitochondrial morphology of respiratory defective cells

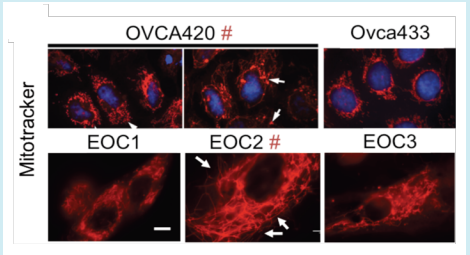


Figure 2. The cells with compromised mitochondria function (#) display aberration in mitochondria morphology with areas of hyperfusion.

Mitochondrial dysfunction is associated with expression of a low MW variant of the Fission Protein Drp1

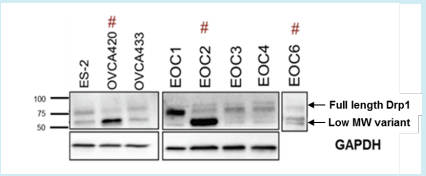


Figure 3. Drp1 protein expression in cell lines and ascites-derived samples indicated expression of a short Drp1 splice variant in mitochondria-defective cells (#).

Expression of Drp1 variants is dependent on anchorage and spheroid formation

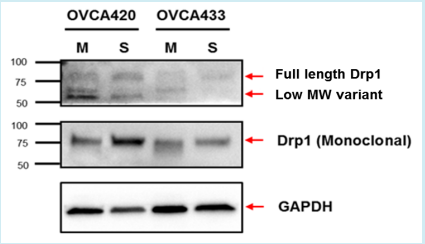
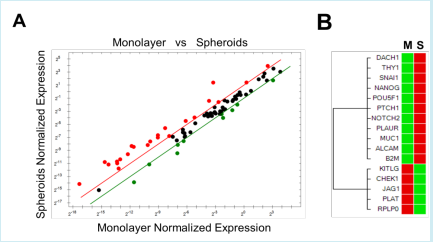


Figure 4. Expression levels of full length Drp1(~82KDa) increase and levels of low molecular variant (~60KDa) decrease when ovarian cancer cells are cultured as anchorage-independent spheroids (S) compared to attached monolayer cultures (M).

Anchorage independent spheroids are associated with full length Drp1 expression and Cancer Stem Cell Markers



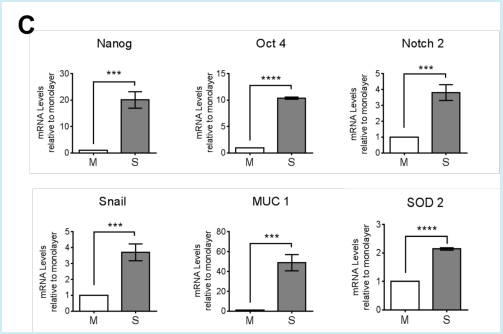


Figure 5. Gene expression levels of cancer stem cell (CSC) markers in OVCA 420 spheroid (S) compared with OVCA 420 monolayer (M). 84 genes involved in CSC were determined using Human Stem Cell RT2 profiler PCR Array (A). Green squares represent up-regulation and red squares represent down-regulation based on at least 2 fold difference (B). Up-regulated genes identified by PCR Array experiments were verified by an independent quantitative RT-PCR (C).

Conclusions

1. Mitochondrial dysfunction may be a hallmark of a subgroup of high grade serous adenocarcinomas.
2. During attachment expression of short Drp1 may act as a Dominant Negative (DN) to inhibit mitochondrial function and alter tumor metabolism towards glycolysis.
3. CSCs may have the ability to up-regulate full length Drp1 to maintain high mitochondria fidelity in anchorage independence.

Future Direction

1. Identification of Drp1 variants in Ovarian Cancer and correlation with mitochondrial dysfunction (RNA sequencing and Mass spectrometry), and patient outcome.
2. Mechanisms of low MW Drp1 expression and function as a Dominant Negative, and the dependence on the tumor microenvironment (ie nutrient availability)

References

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4. Boland, M.L., Chourasia, A.H. & Macleod, K.F. Mitochondrial dysfunction in cancer. *Frontiers in oncology* **3**, 292 (2013).
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Funding: NIH/NCI: R00CA143229